SLC transporters and drug discovery: unlocking the "gatekeepers" as therapeutic targets for rare diseases

Lucia Azzollini [1], Francesca Sassone [1], Gernot Wolf [2], Alvaro Ingles-Prieto [2], Viviana Agus [1], Loredana Redaelli [1], Lia Scarabottolo [1]

1 Axxam S.p.A., OpenZone, Bresso, Milan, Italy
2 CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

The SLCs crucial role as a target for drug discovery

The **solute carrier proteins (SLCs)** comprise a **superfamily of transporters** controlling the import and export of molecules across membranes thus playing essential roles in a multitude of physiological and pharmacological processes. SLCs are the largest family of transporters encoded by the human genome (**more than 450 members**)**.** Genetic studies have evidenced that **a perturbation of SLC transporters' function underlies numerous human common and rare diseases**, thus making **SLC transporters attractive drug targets**. Despite this, SLCs have been long under-explored and indeed **less than 5% of all the SLC transporters are currently targeted by approved drugs**.

In the context of Resolute, **Axxam** has contributed, by mean of its consolidated experience and expertise, in the **development of functional cell-based assays for many different SLCs**, applying multiple and diversify technologies including **fluorescent dyes- and substrates-, genetically encoded sensors-, optogenetic-, radiometric- and imaging- based detection methods.**

The **development of SLC functional assays suitable for high-throughput screening campaigns** represents a fundamental approach to **discover novel inhibitors and activators of SLC transporters for therapeutic purposes.**

Resolute: Research Empowerment on Solute Carriers (SLCs)

RESOLUTE is an academic-industry IMI partnership with the main goal to create a decisive advancement in the overall **tractability of the Solute Carrier class of protein transporters (SLCs), for medical research and development,** by providing practical and conceptual advances, and making its research output available openly and pre-competitively to the scientific community. **Axxam is a member of RESOLUTE partnership to tackle SLCs.**

SLCs case studies for rare diseases

In this poster we are focusing on some **SLCs case studies whose mutations and loss-of-function are crucial for rare diseases onset and progression.**

The first case study is **SLC6A8** whose frameshift and splicing mutations induce **X-linked creatine transporter deficiency,** a rare X-linked single gene disorder.

The second case study is **SLC26A9** whose rare loss-of-function mutations are involved in **Idiopathic diffuse Bronchiectasis**, a chronic lung disease that **resembles cystic fibrosis i**n many aspects.

For both we have developed in Axxam **robust cell-based assays suitable for running HTS** by using different **detection systems, i.e. Membrane Potential dye and SuperClomeleon Chloride biosensor**.

Axxam assay quality criteria for screening purpose

To be suited for a High Throughput Screening (HTS), **an assay, for a cell line recombinantly expressing the SLC of interest,** must satisfy some fundamental quality parameters:

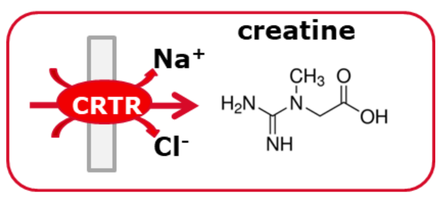
- Miniaturization at least in **384 well plate format**
- **Specific response to the substrate**
- **Negligible response in mock** transfected or non-induced cells
- **Significant signal to background window, displaying a proper RZ' factor (≥0.4)**
- **Signal stability** over culture passages
- **DMSO presence well-tolerated**
- **Pharmacology reproducibility** in independent experiments.

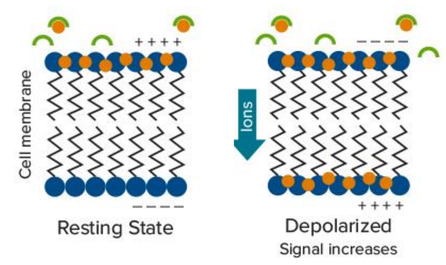
HEK-293 CELL LINE STABLY EXPRESSING SLC6A8 IN INDUCIBLE SYSTEM

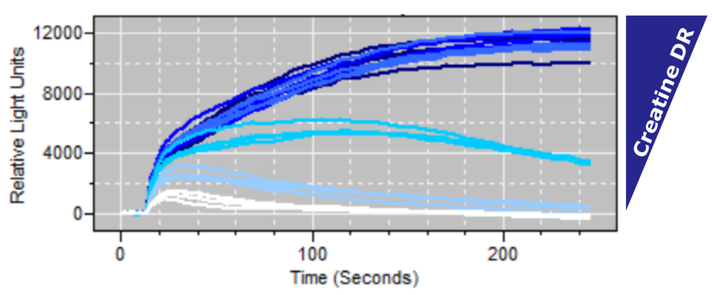
SLC6A8 (CRTR, creatine transporter) is a **Na^+/Cl^- coupled electrogenic cotransporter**, belonging to GABA subgroup of SLC6 family, **mediating the creatine uptake** into a variety of cells.

Creatine is an essential metabolite for the storage and rapid supply of energy in muscle and nervous cells. **In humans, impaired metabolism, transport and distribution of creatine** throughout tissues **can cause varying forms of mental disability,** also known as **creatine deficiency syndrome (CDS).**

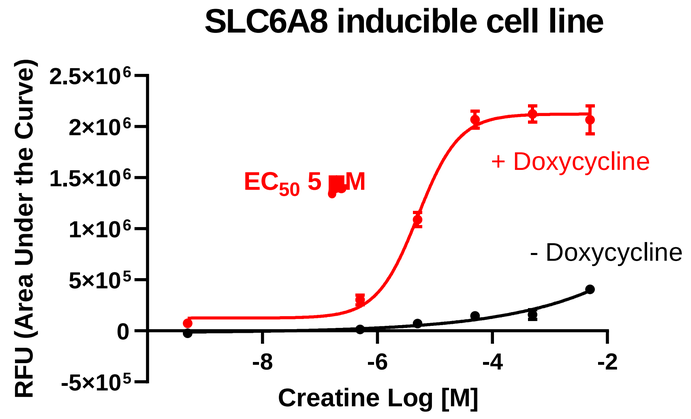
The net-positively charged ions flux inside the cell by SLC6A8 induces a **depolarization of membrane potential** and consequently an **increase of fluorescent signal detected by membrane potential dye.**

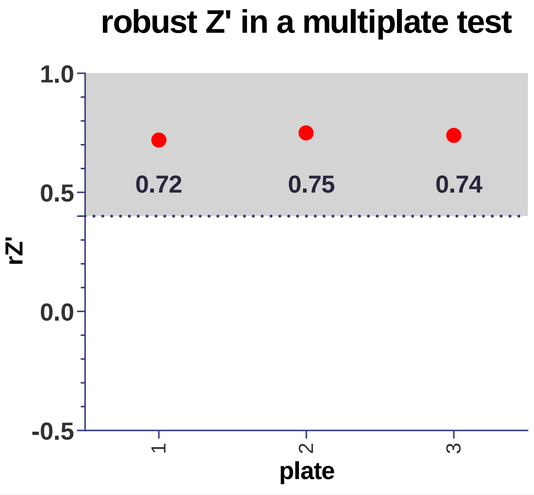






Membrane potential depolarization kinetic traces at FLIPR Tetra fluorescent reader

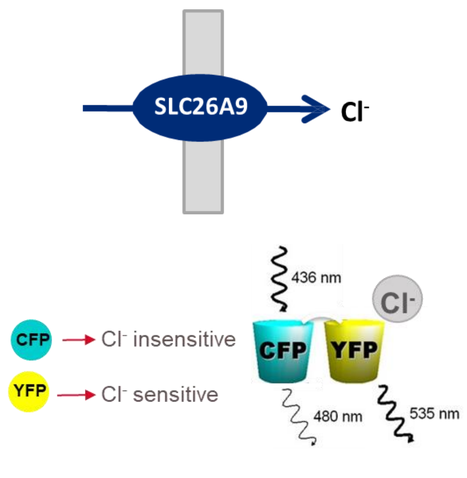




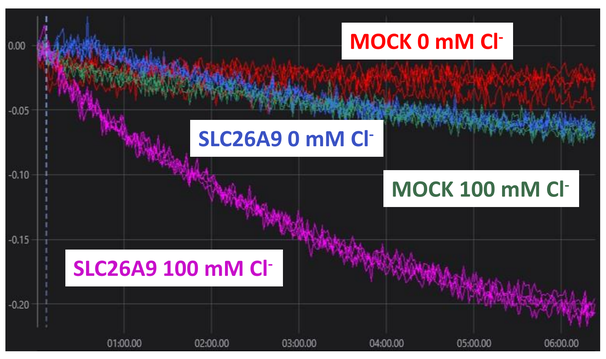
HEK-293 CELL LINE STABLY EXPRESSING SLC26A9 AND TRANSIENTLY EXPRESSING THE SUPERCLOMELEON BIOSENSOR

SLC26A9 is a **highly selective Cl^- transporter**, Cl^-/HCO_3^- exchanger and possibly a Na^+-anion cotransporter. **SLC26A9 functionality is strictly associated with CFTR** (the cystic fibrosis transmembrane conductance regulator).

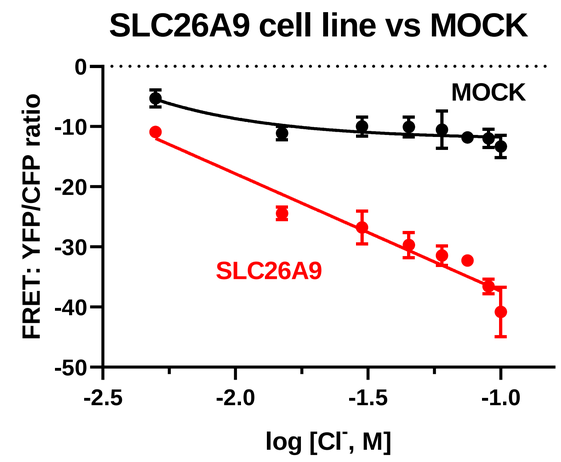
The use of the **SuperClomeleon biosensor** (high Cl^- sensitivity) allows the **ratiometric intracellular chloride measurements** since the binding of chloride to YFP quenches fluorescence emission altering fluorescence resonance energy transfer (FRET) between the CFP donor and the YFP acceptor.

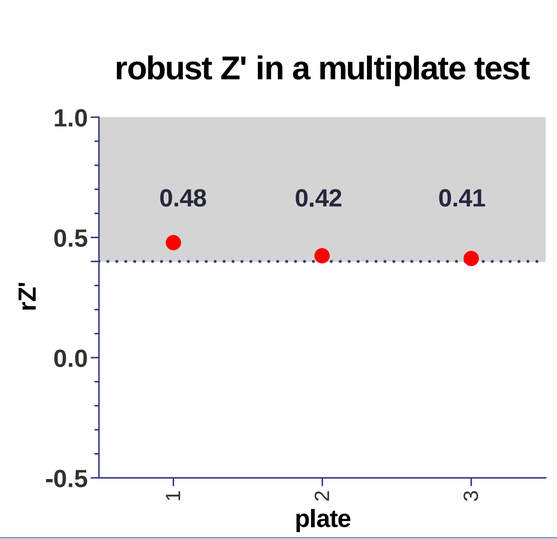


FRET Ratio YFP/CFP (535nm/480nm) **decreases upon Chloride binding**



SuperClomeleon kinetic traces at Hamamatsu FDSS 7000 reader





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